

The Osmotic Potential of Polyethylene Glycol 6000¹

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BURLYN E. MICHEL AND MERRILL R. KAUFMANN

Department of Botany, University of Georgia, Athens, Georgia 30601 and Department of Plant Sciences, University of California, Riverside, California 92502

ABSTRACT

Osmotic potential (ψ_s) of aqueous solutions of polyethylene glycol 6000 (PEG-6000) was curvilinearly related to concentration. At given concentrations, ψ_s increased linearly with temperature. The effects of concentration and temperature on ψ_s of PEG-6000 solutions differ from those for most salts and sugars and apparently are related to structural changes in the PEG polymer. Measurements of ψ_s with thermocouple psychrometers are more negative than those with a vapor pressure osmometer, with the psychrometer probably giving the more nearly correct ψ_s for bulk solutions. An empirical equation permits calculation of ψ_s from known concentrations of PEG-6000 over a temperature range of 15 to 35 C. Viscometry and gravimetric analysis are convenient methods by which the concentrations of PEG-6000 solutions may be measured.

The use of polyethylene glycol to adjust osmotic potential (ψ_s) requires accurate knowledge of the effect of PEG² on ψ_s . The calculation of ψ_s from freezing point depression is unsatisfactory (9). Thermocouple psychrometry (9, 11, 13, 19-21) and vapor pressure osmometry (7, 12, 17) have been employed; however concentrations used to achieve ψ_s values frequently have not been given (7, 11-14, 17) or were reported graphically (4, 9, 19-21) so that accurate numerical values cannot be obtained. Until 1969 the only published data for PEG-6000 came from Zur (4, 20, 21), who expressed concentration as g/1000 cc, without further specification. In 1969, data partially obtained from Zur appeared (19) which indicated that his original expression meant g/1000 cc H₂O. Because the latter is neither molar nor, correctly, molal (6), this interpretation had not been obvious; and some confusion (7) and error (16) had occurred.

Our results show marked discrepancies between thermocouple psychrometry and vapor pressure osmometry, unusual temperature effects, and synergism between PEG and other solutes. We have attempted to evaluate thermocouple psychrometry and vapor pressure osmometry for use with PEG solutions, to establish concentration-temperature- ψ_s relationships, to compare our results with those of others, to test gravimetric and viscosity methods for assaying PEG concentrations, and to elucidate the molecular basis for concentration, temperature, and synergistic effects. PEG-6000 was chosen be-

cause it has been considered better for work with plants than PEGs of lower molecular weight (8, 14).

MATERIALS AND METHODS

Osmotic potentials of PEG-6000 (Carbowax 6000, Union Carbide Corporation) solutions were determined with isopiestic thermocouple psychrometers (2) and two vapor pressure osmometers (Hewlett-Packard Model 302B). No difference was observed between standard and pharmaceutical grades of PEG-6000. Isopiestic measurements of ψ_s were made by placing PEG solutions on discs of filter paper in the bottoms of the psychrometer chambers. The chamber walls were freshly coated with melted petroleum jelly to minimize adsorption of water (1). Following measurements with water on the wet junction, the drop was replaced with a mannitol solution having a potential near that of the PEG (15). Reliable isopiestic measurements could be made at 15, 25, and 35 C. Vapor pressure osmometer measurements were made after calibration with NaCl (10, 18) or mannitol solutions. Either calibration solution and VPOs in both of our laboratories gave equal results. VPO measurements on one instrument could be made over the temperature range of 25 to 65 C.

Because many researchers have observed a distinctly curvilinear relationship between ψ_s and concentration of PEG, and because a temperature rise increases the ψ_s of PEG-6000, we examined the viscosity of PEG over ranges of temperature and concentration to try to determine if the shape of the molecule remained constant. Viscosities were measured with Cannon-Fenske (3) viscometers calibrated with sucrose solutions (6). The viscometers were immersed in a Warburg respirometer refrigerated water bath and were held in a plywood cover with rubber stoppers.³ Observations were through a periscope constructed of two mirrors and Plexiglas.

RESULTS AND DISCUSSION

VPO measurements of ψ_s were consistently higher than those with thermocouple psychrometers (Fig. 1). The test solution in a VPO exists as a small droplet rather than as a pool with large surface area. The possibility that dilution at the droplet surface from condensation of water vapor might produce the lowered response was refuted by using sufficiently concentrated NaCl solution in the wick assembly to force evaporation rather than condensation at the surface and finding no alteration in response.

Although the reason for the differences remains unexplained,

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² Abbreviations: PEG: polyethylene glycol; VPO: vapor pressure osmometer.

³ American Society for Testing Materials, Philadelphia, Penn. ASTM Committee D-2. 1956. Test for kinematic viscosity (D445-53T), Appendix A, in ASTM Standards on Petroleum Products and Lubricants, pp. 201-203.

the following evidence convinced us that psychrometric values of ψ_s for PEG more closely approximate those of bulk solutions than do VPO measurements. When equal solutions (300 g of PEG/kg H₂O) were placed on the ring (2 mm diameter) and in the cup of a psychrometer chamber, the potential of the solution on the ring was effectively 1.5 bars less negative. Similar experiments with water or sucrose solutions on the ring and in the cup demonstrated no apparent differences in potential. The VPO measurement at 25 C for the same concentration was 0.9 bar less negative than indicated by psychrometry. A VPO was modified to permit use of test solutions in bulk rather than as droplets by placing into the sample chamber a filter paperlined aluminum cup holding solution and adding water only to the reference bead after the bridge had been balanced with dry thermistor beads. Solutions of PEG and mannitol with the same ψ_s according to psychrometry produced equal VPO meter readings. This technique is impractical for routine use, but the results obtained reinforced the conclusion that the configuration of the sample as a droplet in the VPO is somehow responsible for the observed error. Finally, cucumber hypocotyl section elongation was inhibited equally by solutions of PEG and mannitol with the same values of ψ_s as determined by psychrometry (14).

A detailed examination of concentration and temperature effects on ψ_s with both techniques is shown in Figure 1. An increase in concentration or a decrease in temperature resulted in more negative values of ψ_s , regardless of technique. To facilitate the use of data shown in Figure 1, an empirical equation was derived for calculating the concentration-temperature- ψ_s relationships. Only psychrometric values of ψ_s were used, and linearity between ψ_s and temperature for given concentrations was assumed. The data were found to fit the following equation:

$$\psi_s = -(1.18 \times 10^{-5}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2 T \quad (1)$$

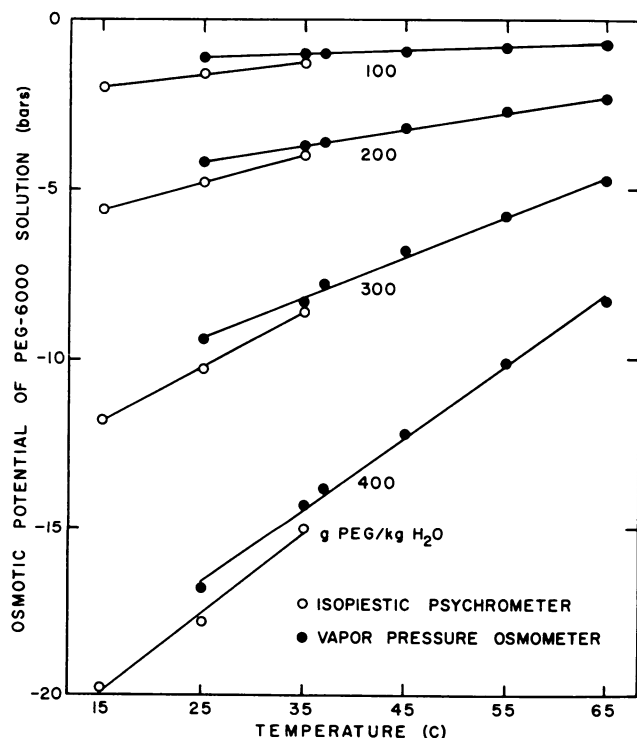


FIG. 1. Effects of temperature and concentration on solute potential of PEG-6000 measured by two techniques.

Table I. Viscosities of PEG-6000 Solutions as Affected by Temperature and Concentration

Temperature C	g PEG-6000/kg H ₂ O				
	0	100	200	300	400
5	1.519	8.18	21.93	46.36	83.38
15	1.139	5.77	15.12	31.39	55.41
25	0.8904	4.34	11.10	22.58	39.48
35	0.7194	3.32	8.35	16.90	29.24
45	0.5960	2.61	6.52	13.01	22.19
55	0.5040	2.12	5.22	10.32	17.53

where C is the concentration of PEG-6000 in g/kg H₂O and T is the temperature in degrees C. For single psychrometric observations, the standard error of estimate for ψ_s from equation 1 was ± 0.28 bar. For specific values of ψ_s and T , equation 1 becomes a simple quadratic and C may be obtained as the positive value in a quadratic solution.⁴

Mean values of ψ_s from thermocouple psychrometry obtained by us and from others (19, H. D. Barrs—personal communication) differ by no more than 0.3 bar for concentrations of PEG-6000 to 250 g/kg H₂O and, except for one anomalous low value (19), by no more than 0.5 bar to 400 g/kg H₂O. Our values are more negative at low concentrations and less negative at high concentrations, the crossover occurring at 200 to 250 g/kg H₂O. The sources of disagreement are not known; however variability in polymer range from one lot of PEG-6000 to another should not be involved because equal weights of PEG-6000 and PEG-20,000 have equal effects on ψ_s , at least from 0 to -10 bars, where sufficient determinations were plotted to justify a definite conclusion (19).

During use a solution's concentration may change. Determination of PEG content and, thus ψ_s , by psychrometry may be impossible, inconvenient, or too time consuming. Viscometry offers a simple, rapid, and accurate method for measuring PEG content of solutions. Graphical or mathematical interpolations for intermediate values may be obtained from the data presented in Table I. Although we have not used them, refractive index (9, 20) and density (20) have been reported of value for measuring rapidly the concentration of PEG. If time is not a factor, perhaps the most accurate method of determining PEG content of an unknown concentration is gravimetric. In a trial, 5 ml each of solutions ranging from 100 to 400 g of PEG/kg H₂O were placed in weighing bottles, weighed, and heated repeatedly at 105 C in a forced-draft oven. Within 4 hr 100 and 200 g of PEG/kg H₂O had reached constant weight. By 6 hr 300 g of PEG/kg H₂O had, but the 400 g/kg had only approached constancy. After a total of 14 hr, the low concentrations had not lost additional weight and the highest concentration had reached stable weight. For 16 samples, four of each concentration, the average was 99.88% of calculated weight, with the greatest deviation being 0.10%. Although approximately 0.1% of the original air-dry PEG-6000 was volatile, the reproducibility far exceeded that expected of any other analytical procedure known to us.

Frequently PEG solutions are prepared with nutrient media instead of pure water. While an analysis has not been made with psychrometry, standard VPO measurements have been made using the No. 2 nutrient solution of Hoagland and

⁴ A table of concentrations required for specific ψ_s values to -20 bars from 15 to 35 C is available from either author.

Arnon (5), various single salts, and several concentrations of mannitol. The VPO data indicate that the ψ_s values of PEG plus other solutes are more negative than the sums of the individual values. With nutrient solution, the synergistic effect was consistently near 7.4% of the ψ_s for PEG in pure water, *e.g.* -0.8 bar nutrient solution plus sufficient PEG to give -8.0 bars in pure water measured -9.4 bars, which is (-8.0×0.074) more negative than -8.8 . To what extent these results reflect idiosyncrasies of the VPO has not been determined. Thermocouple psychrometrically measured synergism between PEG-20,000 and ions has been reported (9). Although we are unable to report useful data, all indications are that the synergistic effect of solutes on the ψ_s of PEG solutions is large enough to be of concern and warrants further investigation by anyone combining nutrients and PEG.

The viscosity data (Table I) were obtained primarily to try to relate molecular configuration to concentration and possibly explain the curvilinear relationship between ψ_s and PEG concentration. To determine the relative effectiveness of successive increments of PEG-6000, for which a concentration change might induce a change in shape, a comparison was made with mannitol, sucrose, and inulin, the shapes of which should not be influenced by concentration. For the latter three, plots of viscosity *versus* molal concentration on semilogarithmic paper give straight lines, *i.e.* viscosity is exponentially related to concentration. A similar plot for PEG-6000 produces a curve that is concave downward; thus each successive increment is relatively less effective in producing viscosity increase than the previous increment. The drop in effectiveness is most pronounced at low concentrations. Such comparisons indicate the PEG molecule to be more stretched out, with greater end-to-end distance, at low than at higher concentrations. Apparently the molecules fold more as concentration increases. In contrast, each added increment of PEG-6000 has greater effectiveness in lowering ψ_s (Fig. 1 and equation 1). Why should the effectiveness of PEG molecules in reducing ψ_s be directly correlated with the degree of molecular folding?

An answer was provided by Dr. G. J. Safford (Union Carbide Corporation, personal communication). Briefly, his findings indicate that in aqueous solution PEG-6000 molecules exist as rather rigid helical segments disrupted occasionally by disorder and folding. While a somewhat ordered cage of water exists around each segment, most hydrogen bonding of water occurs to ether oxygens of PEG exposed where the helix is disordered. In dilute solutions, disorder is minimal and the osmotic effect per molecule is small, whereas in concentrated solutions disorder and osmotic effect are large. Apparently, the synergistic effect of salts we noted above is caused by an alteration of the cage structure of water around the helical seg-

ments of PEG so more water can hydrogen bond to the polymer. Increased folding of the PEG molecules in the presence of salts and mannitol is indicated by reduced relative viscosities. The temperature effect on ψ_s probably is complex, but hydrogen bonding between PEG and water must be reduced as temperature increases.

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